UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
National Health and Environmental Effects Research Laboratory
Research Triangle Park, NC 27711

OFFICE OF RESEARCH AND DEVELOPMENT

February 26, 2019

Dr. Brent Morse
Director of Compliance and Oversight
Office of Laboratory Animal Welfare
Rockledge One, Suite 360
6705 Rockledge Drive, MSC-7982
Bethesda, MD 28092-7982

Dear Dr. Morse;

The purpose of this letter is to describe an animal welfare issue at our institution (OLAW Assurance number; A4051-01) which was identified on Friday November 2, 2018. Your office was given a preliminary notice of this issue via telephone contact to you by our IACUC Chair (b) (6) on November 6, 2018. We would like to inform your office of the IACUC's disposition of the issue.

The animal care contractor animal facility manager (AFM) received an alarm notice from the zebrafish rack alarm system at approximately 10 PM on November 1, 2018. Upon receiving the alarm, the AFM went into the facility. The housing system in alarm consists of five interconnected fish racks. The institution houses additional stand-alone fish racks. The AFM noted that the alarms did not convey the extent of the issue. She found a leak around a pipe at one of the interconnected rack's sump tank. She consulted the rack manufacturer. After several unsuccessful attempts to fix the leak, as recommended by the manufacturer, she was told that the manufacturer recommended shutting down the electricity to the interconnected racks. She shut down the electricity as recommended at 1 AM on November 2, 2018. There were about 83 zebrafish-occupied tanks on the interconnected five-rack system, with approximately 2,000 zebrafish affected.

The attending veterinarian (AV) and the research point of contact were informed the following morning, Friday, November 2, 2018, of the leak and subsequent shutdown of the electricity. With the arrival of more staff and the manufacturer's technical service representative, the electricity had been turned on to the interconnected racks again, and the leak was active. Both the researcher and AV disagreed with the manufacturer's recommendation to shut off the electricity to the rack for 6 hours as this would affect not only the zebrafish but also the nitrifying bacteria needed to break down the ammonia in the water. There was discussion between the manufacturer's representative, the animal program director, the AV, the researcher, and the AFM. It was decided to move the youngest fish (about 1,325 zebrafish in 53 tanks) from the

interconnected system to stand-alone racks in hopes of keeping all the fish alive. The system was shut down again and the leaking sump then drained in order to fix the sump leak.

Most fish were moved to two other functioning racks--one of which already housed healthy fish, whereas the other was just coming on line and did not yet house any fish. Water quality testing of the sumps and three rack systems involved was not conducted until several hours after fish were moved to new racks. Water quality testing continued on two tanks per rack and the sump of each rack once a day during the weekend. Water quality parameters were not ideal on the first day, November 2; however, results were within acceptable parameters on Monday, November 5, 2018. Water quality testing continued once or twice daily during the remainder of the week of November 5, 2108, when we expect ammonia spikes due to increased fish numbers on new racks, and possibly on the interconnected racks and values were a little high or normal on all affected racks.

The IACUC chair was notified of the event on Monday November 5, 2018. On November 5, 2018, no fish had been found dead or otherwise affected from the equipment failure. However, as ammonia spikes are expected, the AV, researchers, and animal care staff will continue to monitor. Breeding success, healthy embryo yield, and research were affected by the event. The week of November 5-9, 2018 embryo numbers were about 50% less than average and embryo quality was also decreased. The next week November 12-16, 2018 embryo numbers were 25% less than average.

The manufacturer recently notified us that the sump came from a bad batch of sumps with defective glue. New sumps were shipped, and the sump was replaced on February 15, 2019. During sump replacement, air pumps and air stones maintain sump water oxygenation to keep the biofilters alive. In the future, if the power is interrupted or if a sump needs to be drained, the portable portions of the biofilters will be moved to an alternate holding area where the water will also be oxygenated.

Toward preventing recurrences and minimizing effects of future equipment failures, the AFM and animal program director will:

- Update checklists and water quality log sheets for each rack. These sheets will be readily available for review by AV, animal care staff, researchers, and the IACUC on each animal room door.
- Write an emergency procedure document clearly delineating the recommended course of events in the case of future major zebrafish equipment failure.
- Provide the IACUC with copies of the updated documents, log sheets, and operating procedures for review and record keeping purposes.
- Order appropriate equipment so that racks can be isolated if there is a future emergency, water quality testing can occur easily, and aeration can be provided to the sensitive sump bacteria.

Both the animal care staff and scientific staff have been reminded that, in the future, the AV should be notified if there is an equipment failure that has the potential to affect animal welfare, as soon as the issue is detected.

We hope these measures satisfactorily address the issue. Please contact (b) (6) (b) (6) or (b) (6) if you have any questions or comments.

Sincerely,

(b) (6)
(b) (6) MD
Director, NHEERL
Institutional Official

(b) (6)
(b) (6) PhD
NHEERL IACUC Chair

cc:
(b) (6) Attending Veterinarian
(b) (6) Director of Animal Resources and Research Support
AAALAC, International